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CYTOTOXIC ACTION OF THE VACCINE AND THE KOKTIGEN TO THE CHICKEN FIBROBLAST IN VITRO

by

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INTRODUCTION

Many investigations have been made in the field of vaccinization and its application as a prevention against infectious diseases. With the improvement of chemotherapeutics, the bacterial infectious diseases such as typhoid fever and dysentery have markedly decreased in the last ten years. But recently, the bacterial infections by the chemotherapetics resistant bacteria are increasing, and this phenomenon is going to be a serious problem in the public health. It is necessary to prevent the infectious diseases both by drugs and the natural and aquired resistance in the body.

This paper deals with the toxic action of heat-killed bacterial vaccine at 60 °C and 100°C to the chicken fibroblast in vitro.

Materials and Methods

The first experiment

Preparations of heat-killed bacterial solutions :

Salmonella. typhosa H. 901 strain of the Microbiological laboratory was used. The bacterial colonies which had been incubated on the nutrient agar during the period of eighteen or twenty hours were collected with the standard platinum string (int he inner volume of its ring equivalent to five mg. of bacteria in wet weight). This bacterial mass was dissolved in Hanks solution (pH 7.4), filtered through gauze into uniform and difuse bacterial suspension. These suspensions were heated in a water bath at a temperature of 100°C and 60°C each.

In the preparation of bacterial filtrate, these heated bacterial suspensions were centrifuged at 3000 r.p.m. for 30 minutes and its supernatant were used.

From eggs incubated for ten days, the chick embryos were removed aseptically and their hearts were cut into small pieces $1 \times 1 mm^3$, arranged three in a row on each cover slide ($22 \times 40 mm$), then inserted into each test tube. These test tubes were placed in the thermostat at 37°C for 30 minutes and finally poured into these test tubes with 4 cc of culture media of Hanks solution 80 % and bovine serum 20 %. After incubation of 48 hours at 37°C, 4 cc of the experimental culture media containing 20% bovine serum were exchanged. On the 1st, 3rd, and 5th day after mediae change, the inserted slides were removed from the test tubes and observed by phase contrast microscope. In each different experimental media, five test tubes were prepared.

The experimental culture media were as follows :

1 A). Four cc of Hanks solution 80 %, bovine serum 20 % (Hanks 80 Bs 20) containing heat-killed Salmonella typhosa 1 mg per ml. (100°C for 30 minutes)

1 A'). Four cc of Hanks 80 Bs 20 containing heat-killed Salmonella typhosa 1mg per ml. (60°C for 30 minutes)

1 B). Four cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.1mg per ml. (100°C for 30 minutes)

1 B'). Four cc of Hanks 80 Bs 20 containing Salmonella 0.1 mg per ml. (60°C for 30 minutes)

1 C). Four cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.01 mg (100°C for 30 minutes)

1 C'). Four cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.01 mg (60°C for 30 minutes)

As control, four cc of Hanks 80 Bs 20 was used.

The culture mediae of the bacterial filtrate of each dilution were as follows :

2 A) Four cc of Hanks solution 80% of the supernatant of the bacterial suspension containing 1 25 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and bovine serum 20% (1 mg of Salmonella typhosa per 1 ml of culture media)

2 A') Four cc of Hanks solution 80% of the supernatant of the bacterial suspension containing 1.25 mg of heat-killed Salmonella typhosa at 60°C for 30 minutes and bovine serum 20%.

2 B) Four cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.125 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and Bs 20.

2 B') Four cc of Hanks 80 of the supernatant of the becterial suspension containing 0.125 mg of heat-killed Salmonella typhosa at 60°C for 30 minutes and Bs 20.

2 C) Four cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.0125 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and Bs 20.

2 C') Four cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.0125 mg of heat-killed Salmonella typhosa at 60°C for 30 minutes and Bs 20.

THE SECOND EXPERIMEMT

To estimate the experiment quantitatively, the dispersed and monolayer culture by Youngner method were used.

Ten-day-chick embryos were cut with scissors and this material was trypsinized in Hanks solution containing 0.25 % trypsin of Difco, and the dispersed cells were collected and separated by centrifugation at 1000 r.p.m. for 10 minutes, counted in a cell count with 0.1 % citric crystal violet and finally adjusted 5×10^5 per 2 ml of Hanks 80 Bs 20 containing different amount of heat-killed Salmonella typhosa vaccine with supernatant of the bacterial suspension in test tube by stationary culture method.

On the 1st, 3rd and 5th day after incubation the cultured cells were measured by trypsinization and citric crystal violet method. The culture media which were used in the second experiment was the same as those of the first experiment.

3 A) Two cc of Hanks 80% Bs 20 % containing heat-killed Salmonella typhosa 1 mg. per ml. (100°C for 30 minutes)

3 A') Two cc of Hanks 80 Bs 20 containing heat-kiled Salmonella typhosa 1 mg per ml. (60°C for 30 minutes)

3 B) Two cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.1 mg per ml. (100°C for 30 minutes)

3 B') Two cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.1 mg per ml. (60°C for 30 minutes)

3 C) Two cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.01 mg (100°C for 30 minutes)

3 C') Two cc of Hanks 80 Bs 20 containing Salmonella typhosa 0.01 mg (60°C for 30 minutes)

4 A) Two cc of Hanks 80 of the supernatant of the bacterial suspension containing 1.25 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and Bs 20 (1mg of Salmonella typhosa per 1 ml of culture media)

Fig. 1 Growth curves of 3 A) and 3 A')
Koktigen' : The culture med ia containheat-killed Salmonlla typhosa 1mg per ml. (100℃ for 30 minutes)
Vaccine : The culture media contain heat-killed Salmonella typhosa 1mg per ml. (60℃ for 30 minutes)
This figure of koktigen' does not mean true koktigen.

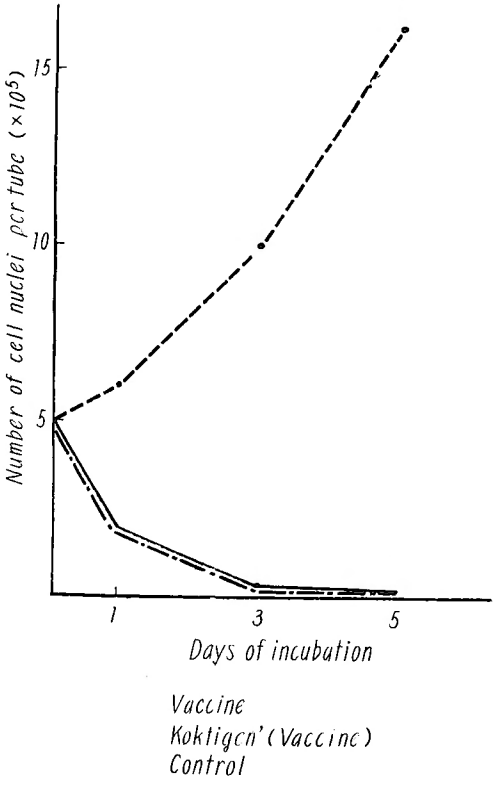
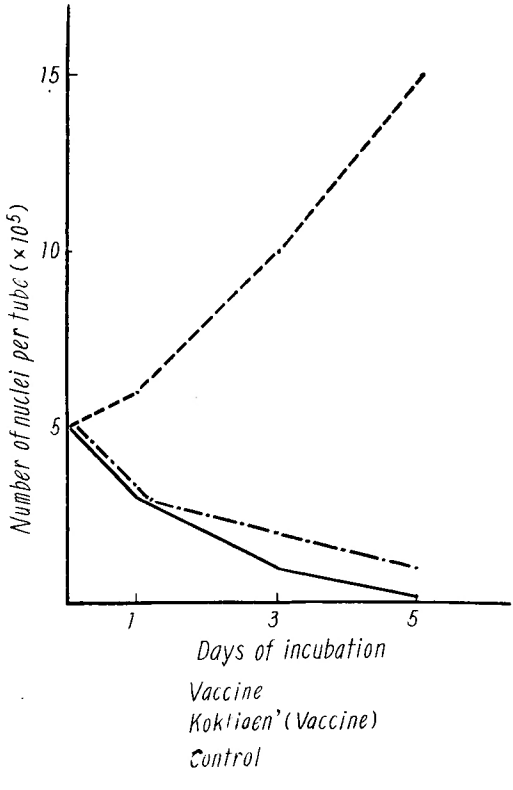


Fig. 2 Growth curve of 3 B) and 3 B')
Koktigen' : The culture media contain heat-killed Salmonella typhosa 0.1mg per ml. (100 ℃ for 30 minutes)
Vaccine : The culture media contain heat-killed Salmonella typhosa 0.1mg per ml. (60 ℃ for 30 minutes)
This figure of Koktigen' does not mean true koktigen.



4 A') Two cc of Hanks 80 of the supernatant of the bactrial suspension containing 1.25 mg of heat-killed Salmonella typhosa at 60°C for 30 minutes and Bs 20.

4 B) Two cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.125 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and Bs 20.

4 B') Two cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.125 mg of heat-killed Salmonella typhosa at 60°C for 30 minutes and Bs 20.

4 C) Two cc of Hanks 80 of the supernatant of the bacterial suspension containing 0.0125 mg of heat-killed Salmonella typhosa at 100°C for 30 minutes and Bs 20.

4' C) Two cc of Hanks 80 of the supernatant of the bacterial suspension containing 0 0125 mg of heat-killed Salmonella Typhosa at 60°C for 30 minutes and Bs 20.

EXPERIMENTAL RESULTS

The first experiment :

Fig. 3 Growth curve of 3 C and 3 C')
Koktigen' : The culture media contain heat-killed Salmonella typhosa 0.01mg per ml. (100℃ for 30 minutes)
Vaccine : The culture media contain heat-killed Salmonella typhosa 0.01mg per ml. (60℃ for 30 minutes)
This figure of Koktigen does not mean true koktigen.

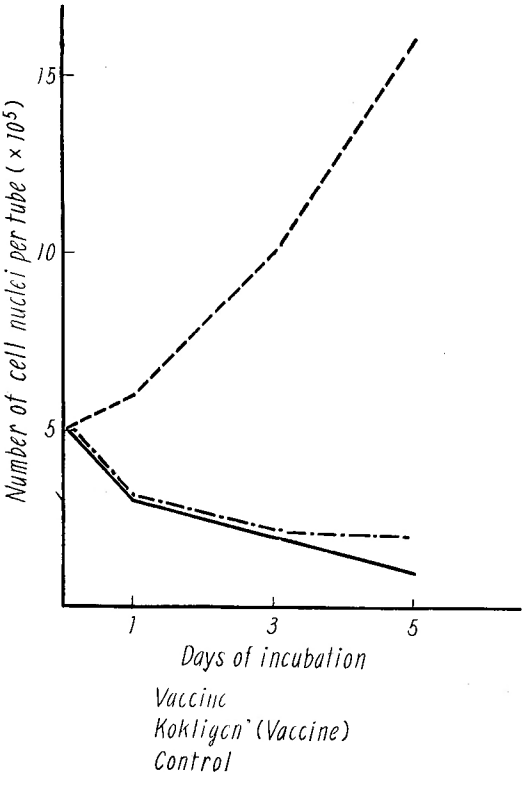
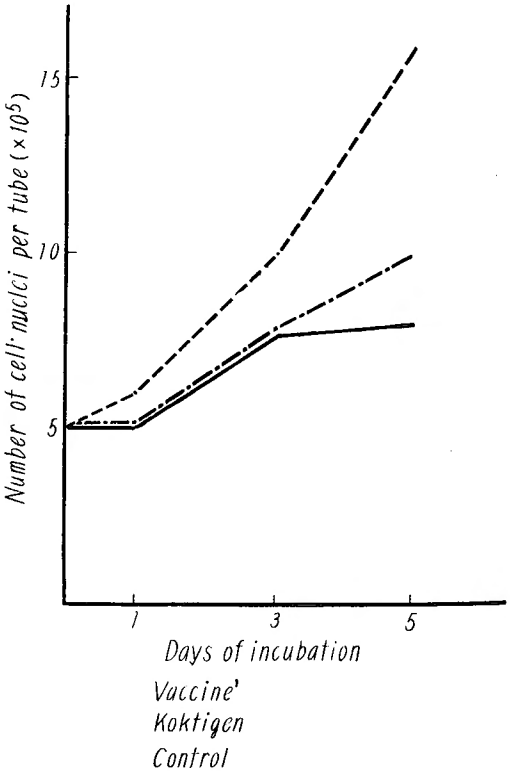


Fig. 4 Growth curves of 4 A) and 4 A')
Koktigen : The culture media consist of the supernatant of the bacterial suspension containing 1mg of heat-killed Salmonella typhosa at 100℃ for 30 minutes per ml.
Vaccine' : The culture media consist of the supernatant of the bacterial suspension 1mg of hea-tkilled Samonella typhosa at 60℃ for 30 minutes per ml.



In the series of bacteria-containing groups, both vaccines in each concentration revealed marked degenerations on the chicken fibroblasts, presenting vacuolization, cytolysis, deformation, and finally total break-down of the cells.

In the comparison with the both vaccine, the toxic action to the fibroblasts, there were no marked differences.

In the experimental series containing no bacteria, 2 A) and 2 A'), 2 B) and 2 B') revealed slight toxic actions to the fibroblasts, but 2 C) and 2 C') revealed no marked differences in the phase contrast microscopy as compared with the control culture.

The second experiment :

In the series of bacteria containing groups, the fibroblasts did not increase in number, but rather decreased in number in the presence of vaccines. In tthe concentrated

Fig. 5 Growth curve of 4 B and 4 B')
Koktigen : The culture media consist of the supernatant of the bacterial suspeusion conaining 0.1mg of heat killed Samonella typhosa at 100°C for 30 minutes per ml.
Vaccine' : The culture media consit of the supernatant of the bacterial suspensian containing 0.1mg of heat-killed Salmonella typhosa at 60°C for 30 minutes per ml.

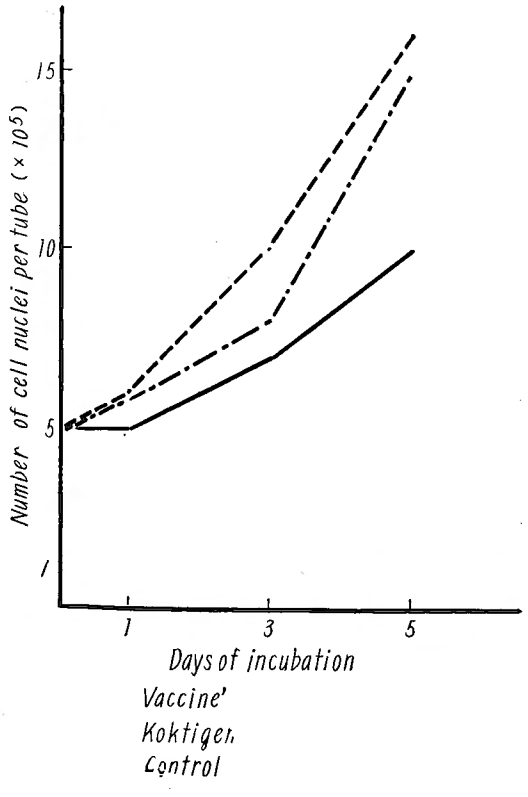
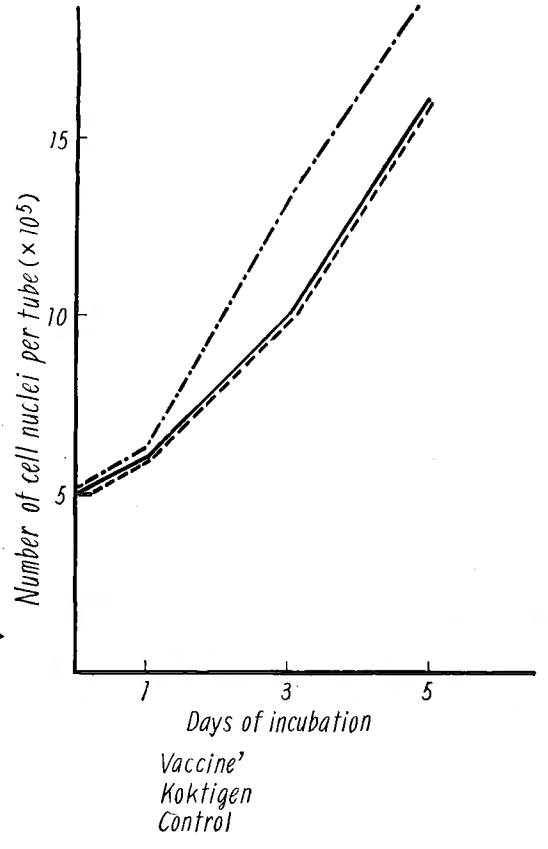


Fig. 6 Growth curves of 4 C and 4 C')
Koktigen · The culutre media consist of the supernatant of the bacterial suspension containing 0.01 mg of heat killed Salmonella typhosa at 100°C for 30 minutes per ml.
Vaccine' : The culture media consist of the supernatant of th bacterial suspension containing 0.01mg of heat killed Salmonella typhosa at 60°C for 30 minutes per ml.



culture media of 3 A), 3 A') and 3 B), 3 B'), the differences between the supernatant of the vaccine and the koktigen was not revealed, but in the dilute concentration of bacterial solutions, the toxic action koktigen were less than those of heated at 60°C for 30 minutes.

In the series of the experiment containing no bacteria, 4 A), 4 A') and 4 B) and 4 B') revealed slight toxic action to the fibroblasts, whereas in the groups of dilute concentration of 4 C) and 4 C') no toxic actions to the fibroblasts were noted, but rather a growth promoting effect.

DISCUSSION

It is considered that Salmonella typhosa has an endotoxin, consisting of phospholipid, polysaccharide, and protein. These components show different immunological behaviors and toxic action. The endotoxin acts as a pyrogen and the bacterial filtrate causes Shwartzman phenomenon which contains cytotoxic action when introduced into animals, causing edema, redness, necrosis in the local site of injection, and hemorrhage in the liver, kidney and bone marrow. The effect of heating on the Salmonella typhosa are assumed to cause the partial inactivation of antigenicity of the bacteria, but the detailed biochemical changes are not known. TORIKATA and his coworkers studied the heat killed bacterial vaccine and koktigen mainly by heating at 100°C for 30 minutes have revealed the most active antibody producing and phagocytic action to the body. In this experiment, it was proved that the supernatant of the heat killed bacterial suspensions had slight toxic action to the chicken fibroblasts but in the dilute concentration it had a favourable efefct to the growth of chiken fibroblast. The fietrates of the bacterial culture suspensions have many enzymes and amino acid and polypeptides. From the biochemical point of view, this requires further investigations.

CONCLUSIONS

This paper deals with the toxic action to chicken fibroblast in vitro. The morphological changes were observed by phase contrast microscope and the toxic action to the growth of the chicken fibroblasts were estimated by the Youngners and citric crystal violet method.

The results obtained are as follows :

1) The heat killed bacterial suspensions of Salmonella typhosa revealed the marked cytotoxic actions to the cultured chicken fibroblasts in the dilute concentration of 0.01 mg per ml of both vaccine and koktigen.

2) The supernatants of the heat killed bacterial suspensions also had slight toxic actions to the chicken fibroblasts in the concentration of 0.1 mg and 1 mg of Salmonella typhosa per ml, but in the concentration of 0.01 mg of Salmonella typhosa per ml of the culture media, the supernatant fluid had a growth promoting action to the cells. In the comparison of vaccine and koktigen, the latter exhibited less toxic actions and much more growth promoting actions to the chicken fibroblasts in vitro than the former.

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Control groups

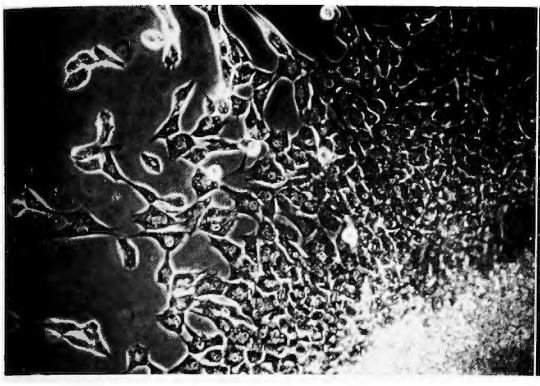

Fig. 1 The chicken fibroblasts of the 3rd day after incubation.

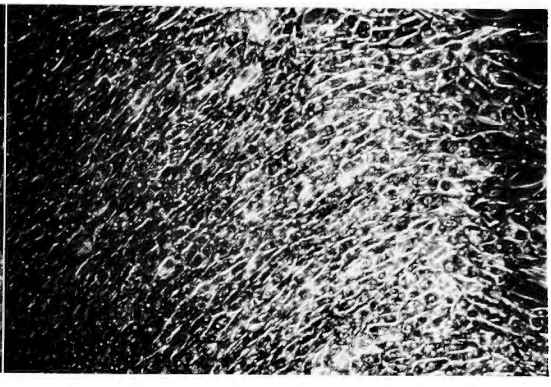

Fig. 2 The chicken fibroblasts of the 5th day after incubation.

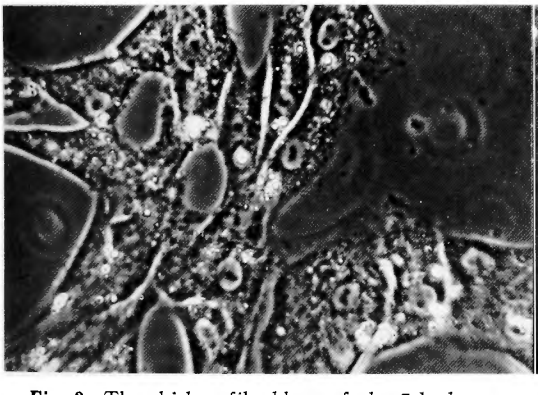

Fig. 3 The chicken fibroblasts of the 5th day after incubation.

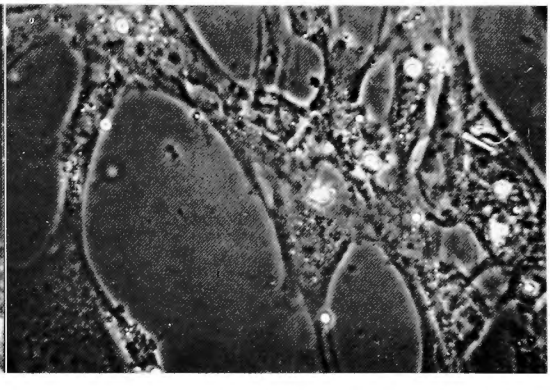

Fig. 4 The chicken fibroblasts of the 5th day after incubation.

The experimental groups of 1 C')

The experimental culture media containing heat-killed Salmonella typhosa 0.01mg per ml (60°C tor 30 minutes)

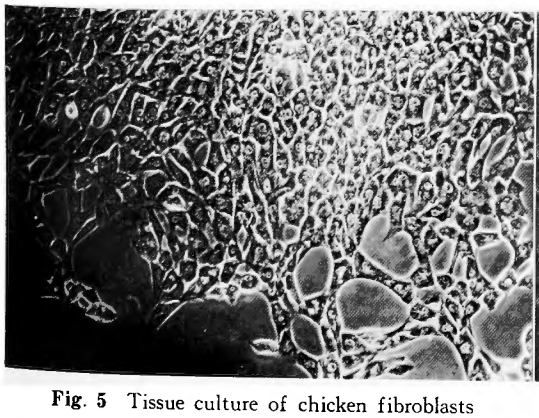

Fig. 5 Tissue culture of chicken fibroblasts before medium exchange.

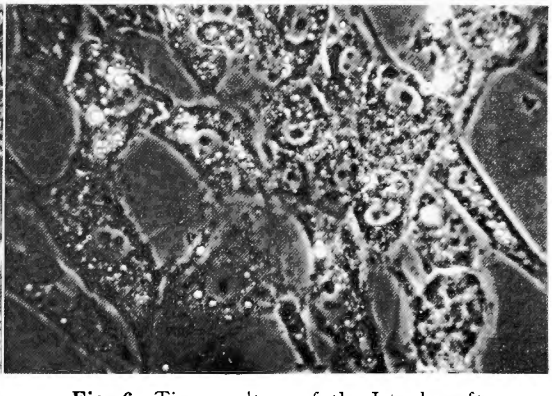

Fig. 6 Tissue culture of the Ist day after adding the experimental medium.

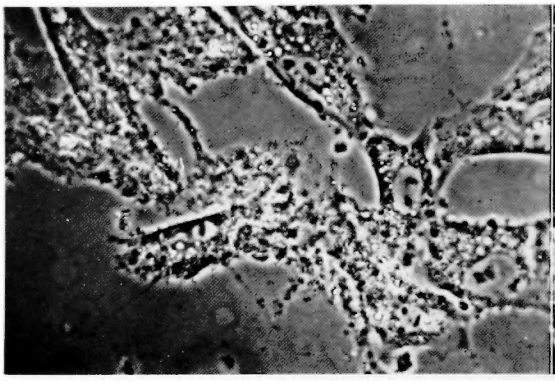

Fig. 7 Tissue culture of the 3rd day after adding the experimental medium.

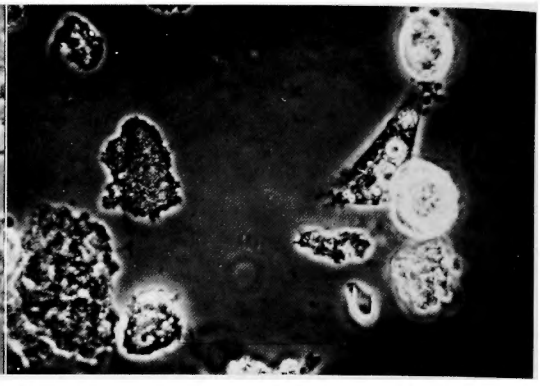

Fig. 8 Tissue culture of the 5th day after adding the experimental medium.

The experimental groups of 1 C)

The experimental culture modia containing heat-killed Salmonella typhosa 0.01mg per ml. (100℃ for 30 minutes)

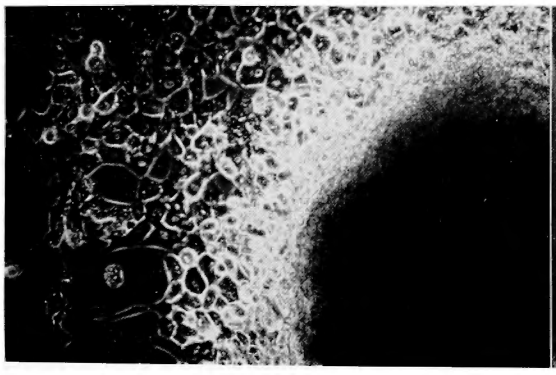

Fig. 9 Tissue culture of chicken fibroblasts before medium change.

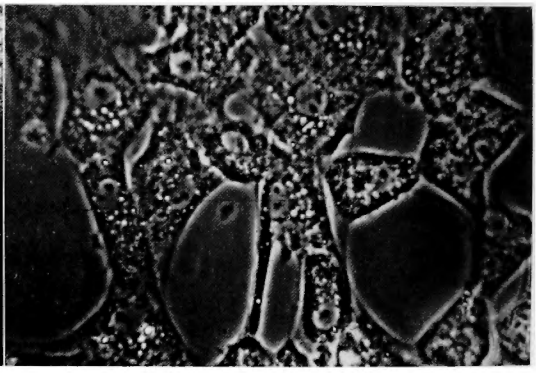

Fig. 10 Tissue culture of the Ist day after adding the experimental medium.

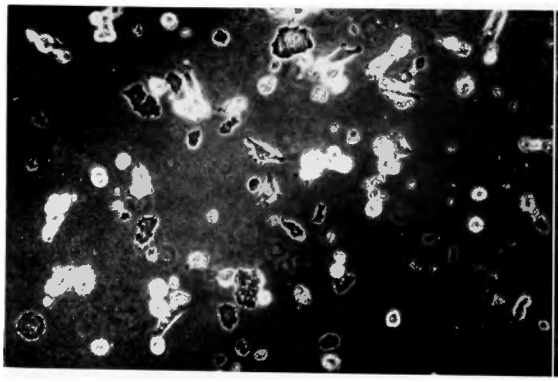

Fig. 11 Tissue culture of the 5th day after adding the experimetal medium.

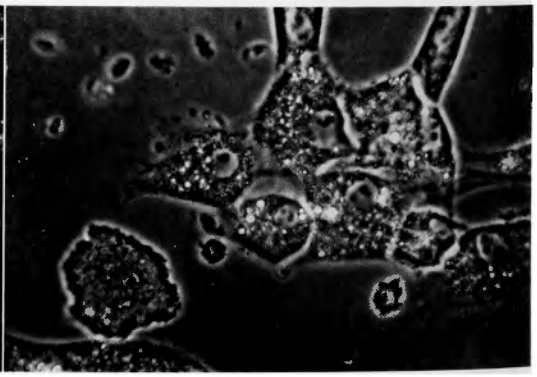

Fig. 12 Tissue culture of the 3rd day after adding the experimental medium.

Control groups

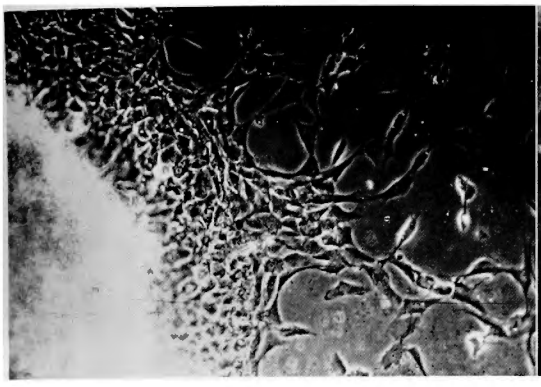

Fig. 13 The tissue culture of the chicken fibroblasts before medium change.

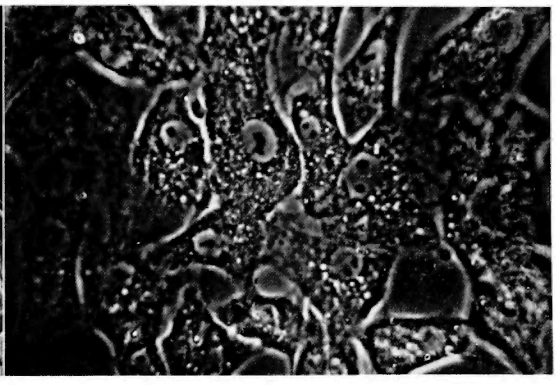

Fig. 14 Tissue culture of the 1st day after medium change.

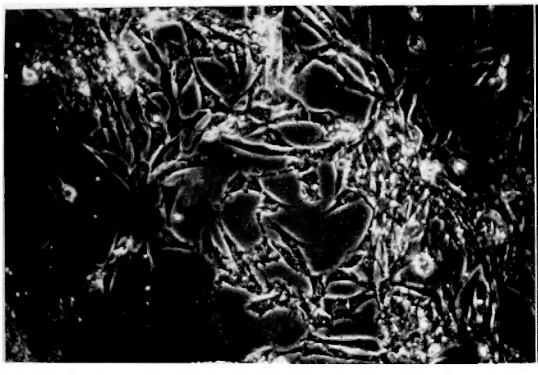

Fig. 15 Tissue culture of the 3rd day after medium change.

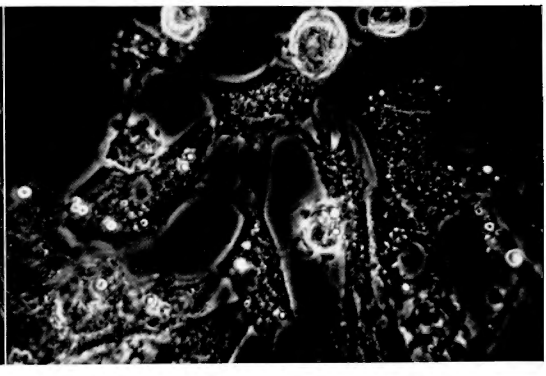

Fig. 16 Tissue culture of the 5th day after medium change.

The experimental groups of 2 C')

The experimental media of the supernatant of the bacterial suspensions containing heat-killed Salmonella typhosa 1mg)60℃ for 30 minutes)

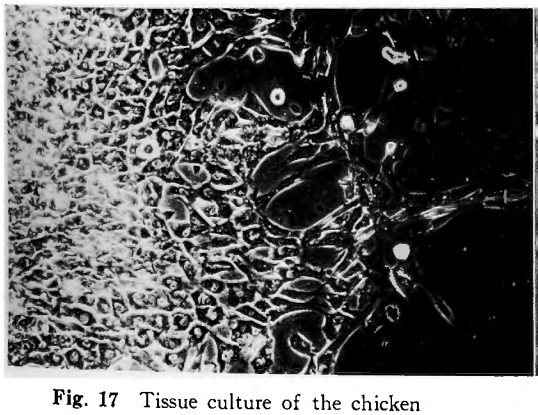

Fig. 17 Tissue culture of the chicken fibroblasts before medium change.

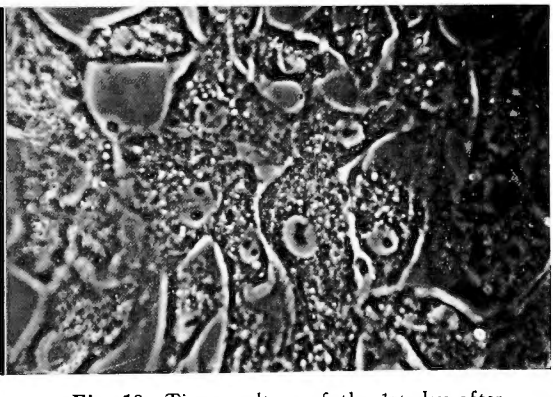

Fig. 18 Tissue culture of the 1st day after adding the experimental media.

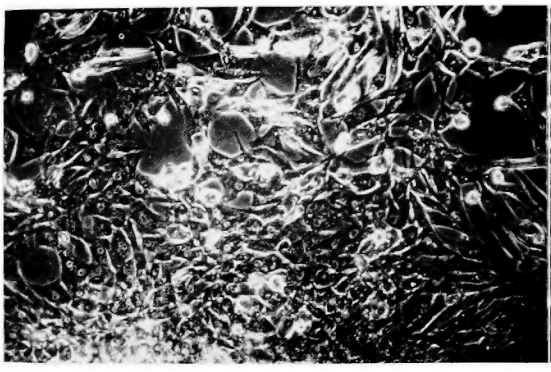

Fig. 19 Tissue culture of the 3rd day after adding the experimental media.

Fig. 20 Tissue culture of the 5th day after adding the experimental media.

The experimental group of 2 A)

The experimental culture media of the supernatant of the bacterial suspensions containing heat-killed Salmonella typhosa 1mg (100℃ for 30minutes)

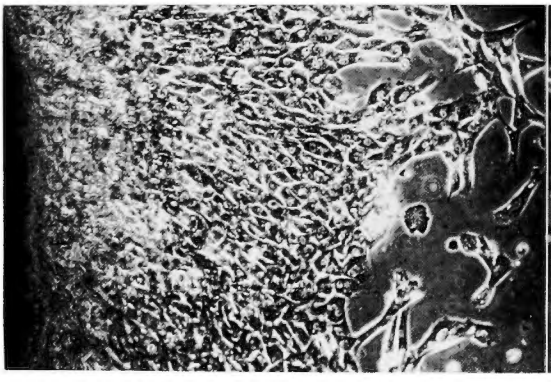

Fig. 21 Tissue culture of chicken fibroblasts before medium change.

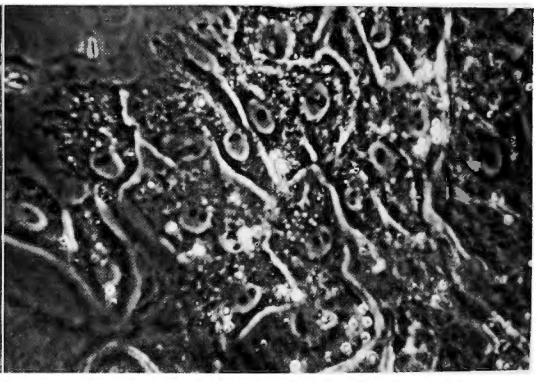

Fig. 22 Tissue culture of the 1st day after adding the experimental culture mediae.

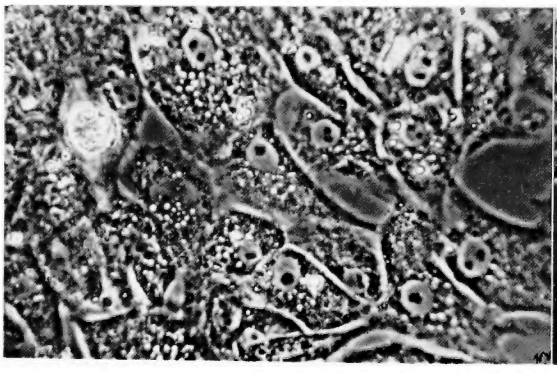

Fig. 23 Tissue culture of the 3rd day after adding the experimental media.

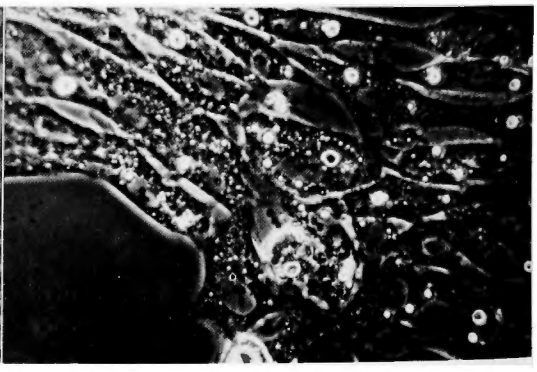

Fig. 24 Tissue culture of the 5th day adding the experimental media.

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和文抄録

培養鶏胎線維芽細胞に対するコクチゲン及びワクチンの毒性について

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孵化後10日の鶏胎児より試験管内スライド培養法によつて増殖した線維芽細胞に対するチフス菌加熱抗原液の毒性を，位相差顕微鏡を用いて観察し，又 Youngner法によつて線維芽細胞の増殖を定量的に追及し次の結果を得た．

1) 菌体を含むチフス菌の 100℃ 30分間，及び60℃ 30分間加熱ワクチンを含むHanks液80% (0.5% Lactoalbumin lydiolysate を含む)，牛血清 20% の培養液は，鶏胎線維芽細胞に対して，1mg/cc，0.1mg/cc 及び0.01mg/ccの濃度に於て著明な毒性を示した．

2) 100℃ 30分間及び60℃ 30分間加熱したチフス菌の菌体を含まない上清成分を加えた培養に於ては，1mg/cc，及び0.1mg/cc の高濃度に於て，鶏胎線維芽細胞に対して軽度の増殖抑制作用を示したが，低濃度に於ては，むしろ細胞増殖促進作用を示した．特にコクチゲンは60℃30分間加熱の菌体を含まない上清成分と比較して，細胞に対する毒性は少かつた．